

CLAIMS:

1. Apparatus for detecting small assayed molecules in a sample, comprising:
 - (a) a sensing member comprising a piezoelectric crystal having at least one sensing surface which can interact with a medium in contact therewith by either binding a first indicator agent from the medium, or by releasing a second indicator agent originally immobilized on the sensing surface into the medium; the medium being either the sample in which case the assayed molecule present, causes the release of the second indicator agent from the at least one sensing surface, or being a treated sample preparation obtained by reacting the sample with one or both of a reagent solution or sample-processing hardware such that said medium comprises a first indicator agent or a second indicator agent-releasing species at a concentration of said agent or species which is in correlation to the concentration of the assayed molecule in the sample, the binding or release resulting in a change of mass of the sensing surface;
 - (b) a testing cell for holding said medium and bringing it into contact with said at least one sensing surface; and
 - (c) an electric or electronic utility for inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof, a change in resonance frequency after contact between the sensing surface and the medium, indicating presence of the explosive in the sample.
2. An Apparatus according to Claim 1, wherein said assayed molecule is an explosive molecule.
3. An apparatus according to Claim 1 or 2, wherein the degree of change in resonance frequency serves as a measure of the level of said assayed molecule in said sample.
4. An apparatus according to Claim 1 or 2, wherein said at least one sensing surface carries capturing agents which bind to neutralizing agents at an assayed molecule-binding domain of the neutralizing agent.

5. An apparatus according to Claim 4, wherein said capturing agents are assayed molecules residues or moieties and said neutralizing agents comprise first antibodies which bind to the assayed molecules.
6. An apparatus according to Claim 2 or 3, wherein said at least one sensing 5 surface carries residues or moieties of the assayed molecules bound to first antibodies which can competitively bind to soluble assayed molecules in a medium in contact with the at least one sensing surface, whereby in the presence of the assayed molecules in said medium the antibodies are released from the at least one sensing surface.
- 10 7. An apparatus according to Claim 5 or 6, wherein the first antibodies are bound or complexed to a mass-increasing agent.
8. An apparatus according to Claim 7, wherein said mass increasing agent comprises a second antibody which binds to said first antibody or comprise avidin or streptavidin bound to a biotin residue conjugated to the first antibody.
- 15 9. An apparatus according to any one of Claims 5-8, wherein said first antibodies are monoclonal antibodies.
10. An apparatus according to Claim 7, wherein said monoclonal antibody has the binding characteristics of the 5B3 monoclonal antibody.
11. An apparatus according to Claim 8, wherein said antibody is said 5B3 20 monoclonal antibody.
12. An apparatus according to any one of Claims 1-11, wherein said assayed molecule is DNT or TNT.
13. A system for detecting small assayed molecules in a sample comprising an apparatus according to any one of Claims 1-12 and one or both of reagents and 25 hardware for processing said sample or for introducing it into said cell.
14. A system according to Claim 13, wherein said hardware comprises a flow system for propelling a medium comprising the sample into said cell.
15. A system according to Claim 13 or 14, comprising:

- (a) a sensing member comprising a piezoelectric crystal having at least one sensing surface which carries residues or moieties of said assayed molecules bound to anti-assayed molecule antibodies;
- (b) a testing cell for holding a medium and bringing it into contact with said at least one sensing surface, whereby in the presence of the assayed molecules in the medium, at least some of said antibodies are released into the medium;
- (c) hardware for introducing the sample into the testing vessel; and
- (d) an electric or electronic utility for inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof, a reduction in resonance frequency after contact between the sensing surface and the medium, indicating presence of said assayed molecule in the sample.

10 16. A system according to Claim 15, wherein said assayed molecule is an explosive molecule.

15 17. A system according to Claim 15 or 16, wherein the anti-assayed molecule antibody is a monoclonal antibody.

18. A system according to Claim 17, wherein the assayed molecule is DNT or TNT and the antibody is that designated herein as 5B3.

19. A system according to any one of Claims 15-18, wherein the anti-explosive antibodies are bound to mass-increasing agents.

20 20. A system according to Claim 19, wherein said mass increasing agent comprises a second antibody, or an avidin or a streptavidin which bind to a biotin moiety conjugated to the anti-explosive antibody.

21. A system according to Claim 13 or 14, comprising:

- (a) a sensing member comprising a piezoelectric crystal having at least one sensing surface which carries residues or moieties of the assayed molecules;
- (b) a reagent system comprising anti-assayed molecule antibodies;
- (c) a testing cell for holding a medium and bringing it into contact with said at least one sensing surface, whereby in the presence of the assayed molecules in the medium, at least some of said antibodies are released into the medium;

(d) an arrangement for contacting the sample with said reagent system to obtain a treated sample preparation and for introducing the treated sample preparation into the testing vessel; and

(e) an electric or electronic utility for inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof, a decrease in resonance frequency after contact between the sensing surface and the treated sample preparation, indicating that the sample is free of the assayed molecules.

5 22. A system according to Claim 20, wherein the assayed molecule is an explosive molecule.

10 23. A system according to Claim 22, wherein the anti-assayed molecule antibody is a monoclonal antibody.

24. A system according to Claim 22, wherein the assayed molecule is DNA or TNT and the antibody is that designated herein as 5B3.

15 25. A system according to any one of Claims 21-24, comprising a mass-increasing agent for binding to said anti-explosive antibodies.

26. A system according to Claim 24, wherein said mass increasing agent comprises a second antibody, or an avidin or a streptavidin which bind to a biotin moiety conjugated to the anti-explosive antibody.

27. A system according to Claim 13 or 14, comprising:

20 (a) a sensing member comprising a piezoelectric crystal having at least one sensing surface which carries capturing agents for binding to neutralizing agents;

(b) a testing cell for holding a medium and bringing it into contact with said at least one sensing surface;

25 (c) a reagent system comprising the neutralizing agents which can bind to the assayed molecules;

(d) an arrangement for contacting the sample with the reagent system under condition and for a time permitting binding of the neutralizing agent to the assayed molecules, to obtain a treated sample preparation;

(e) a filtration system for filtering out from said treated sample preparation neutralizing agents unbound to an explosive molecule to obtain a filtrate essentially devoid of such unbound neutralizing agents;

(f) arrangement for transfer of said filtrate to said testing cell; and

5 (g) an electric or electronic utility for inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof, an increase in resonance frequency after contact between the sensing surface and the filtrate, indicating presence of the assayed molecules in the sample.

28. A system according to Claim 27, wherein the assayed molecule is an explosion molecule.

10 29. A system according to Claim 27 or 28, wherein said neutralizing agent is an anti-explosive antibody.

30. A system according to Claim 29, wherein said antibody is a monoclonal antibody.

15 31. A system according to Claim 30, wherein the assayed molecule is DNT or TNT and the monoclonal antibody is that designated herein as 5B3.

32. A system according to any one of Claims 27-31, wherein said filtration system comprises immobilized residues or moieties of molecules of the assayed molecule.

20 33. A system according to any one of Claims 27-32, wherein said neutralizing agent is conjugated to a moiety which binds to said capturing agents.

34. A system according to Claim 33, wherein said moiety is a biotin residue and said capturing agent is avidin or streptavidin.

35. A system according to Claim 13 or 14, comprising:

25 (a) a sensing member comprising a piezoelectric crystal having at least one sensing surface which carries capturing agents for binding to neutralizing agents;

(b) a testing cell for holding a medium and bringing it into contact with said at least one sensing surface;

(c) a reagent system comprising neutralizing agents which can bind to the assayed molecules, said neutralizing agent being conjugated to an enzyme which can catalyze a reaction yielding an insoluble reaction product;

(d) an arrangement for contacting the sample with the reagent system under condition and for a time permitting binding of the neutralizing agent to the assayed molecules, to obtain a treated sample preparation;

(e) a filtration system for filtering out from said treated sample preparation neutralizing agents unbound to an assayed molecule to obtain a filtrate essentially devoid of such unbound neutralizing agents;

(f) arrangement for transfer of said filtrate to said testing cell;

(g) an ensemble of reagents and conditions for inducing said enzyme to catalyze the reaction yielding the insoluble reaction product; and

(h) an electric or electronic utility for inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof, an increase in resonance frequency after contact between the sensing surface and the filtrate or after permitting the enzyme to catalyze said reaction, indicating presence of the assayed molecule in the sample.

36. A system according to Claim 35, wherein the assayed molecule is an explosive molecule.

37. A system according to Claim 35 or 36, wherein said neutralizing agent is an anti-explosive antibody.

38. A system according to Claim 37, wherein said antibody is a monoclonal antibody.

39. A system according to Claim 38, wherein the assayed molecule is DNT or TNT and the monoclonal antibody is that designated herein as 5B3.

40. A system according to any one of Claims 36-39, wherein said filtration system comprises immobilized residues or moieties of the assayed molecules.

41. A system according to any one of Claims 36-40, wherein said capturing agent is an immobilized antibody which binds a moiety of said enzyme, such binding not interfering with the catalytic activity of said enzyme.

42 A system according to any one of Claims 35-41, wherein said enzyme is selected from the group consisting of horseradish peroxidase, microperoxidase, alkaline phosphatasae, glucoseoxidase and galactosidase.

43. A method for detecting a small assayed molecule in a sample, comprising:

5 (a) providing a sensing member comprising a piezoelectric crystal having at least one sensing surface which can interact with a medium in contact therewith by either binding a first indicator agent from the medium, or by releasing a second indicator agent originally immobilized on the sensing surface into the medium;

10 (b) contacting the at least one sensing surface with a medium being either the sample in which case the assayed molecule if present causes the release of the second indicator agent from the sensing surface, or being a treated sample preparation obtained by reacting the sample with one or both of a reagent solution or sample-processing hardware, such that said medium comprises a first indicator agent or a second indicator agent-releasing species, at a concentration of said agent or species which is in correlation to the concentration of the assayed molecule in the sample, the binding or release resulting in a change of mass of the sensing surface;

15 (c) inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof, and

20 (d) determining whether a change in resonance frequency after contact between the sensing surface and the medium occurred, such change indicating presence of the assayed molecule in the sample.

44. A method according to Claim 32, wherein said small molecule is an explosive.

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45. A method according to Claim 44, wherein said explosive is DNT or TNT.

46. A method according to any one of Claims 43-45, wherein the degree of change in resonance frequency serves as a measure of the level of said molecule in said sample.

30 47. A method according to Claim 43, comprising:

- (a) providing a sensing member comprising a piezoelectric crystal having at least one sensing surface which carries residues or moieties of the assayed molecules bound to anti-assayed molecule antibodies;
- (b) contacting the sample with the at least one sensing surface;
- 5 (c) inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof; and
- (d) determining whether there occurred an increase in resonance frequency after contact between the sensing surface and the medium, such increase indicating presence of the assayed molecule in the sample.

10 48. A method according to Claim 47, wherein the assayed molecules are explosive molecules.

49. A method according to Claim 47 or 48, wherein the anti-explosive antibody is a monoclonal antibody.

50. A method according to Claim 49, wherein the assayed molecule is DNT or

15 TNT and the antibody is that designated herein as 5B3.

51. A method according to any one of Claims 47-50, comprising binding a mass increasing agent to the antibodies.

52. A method according to Claim 51, wherein said mass increasing agent comprises a second antibody, or an avidin or a streptavidin which bind to a biotin moiety conjugated to the anti-assayed molecule antibody.

20 53. A method according to Claim 43, comprising:

- (a) providing a sensing member comprising a piezoelectric crystal having at least one sensing surface which carries residues or moieties of the assayed molecules;
- 25 (b) contacting the sample with anti-assayed molecule antibodies and incubating for a time allowing the antibodies to bind to the assayed molecules if present in the sample, to yield a treated sample preparation;
- (c) contacting the treated sample preparation with the at least one sensing surface;

- (d) inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof; and
- (e) determining whether there occurred a decrease in resonance frequency after contact between the sensing surface and the treated sample preparation, such decrease indicating presence of the assayed molecule in the sample.

5 54. A method according to Claim 53, wherein the assayed molecule is an explosive molecule.

55. A method according to Claim 53 or 54, wherein the anti-assayed molecule antibody is a monoclonal antibody.

10 56. A method according to Claim 55, wherein the assayed molecule is DNT or TNT and the antibody is that designated herein as 5B3.

57. A method according to any one of Claims 53-56, comprising binding a mass-increasing antibody to the anti-explosive antibody.

15 58. A method according to Claim 57, wherein said mass increasing agent comprises a second antibody, or an avidin or a streptavidin which bind to a biotin moiety conjugated to the anti-explosive antibody.

59. A method according to Claim 43, comprising:

- (a) providing a sensing member comprising a piezoelectric crystal having at least one sensing surface which carries capturing agents for binding to neutralizing agents;
- (b) contacting the sample with neutralizing agents which can bind to the assayed molecules under condition and for a time permitting binding of the neutralizing agents to the assayed molecules, to obtain a treated sample preparation;
- 20 (c) filtering the treated sample preparation through filtration system to filter out neutralizing agents unbound to the assayed molecules to obtain a filtrate essentially devoid of such unbound neutralizing agents;
- (d) contacting said filtrate with the at least one sensing surface;
- (e) inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof; and

(f) determining whether there occurred a increase in resonance frequency after contact between the sensing surface and the treated sample preparation, such increase indicating presence of the assayed molecules in the sample.

60. A method according to Claim 59, wherein the assayed molecules are explosive molecules.

61. A method according to Claim 59 or 60, wherein said neutralizing agent is an anti-explosive antibody.

62. A method according to Claim 61, wherein said antibody is a monoclonal antibody.

10 63. A method according to Claim 62, wherein the assayed molecule is DNT or TNT and the monoclonal antibody is that designated herein as 5B3.

64. A method according to any one of Claims 59-63, wherein said filtration system comprises immobilized residues or moieties of molecules of the explosive.

15 65. A method according to any one of Claims 59-64, wherein said neutralizing agent is conjugated to a moiety which binds to said capturing agents.

66. A method according to Claim 65, wherein said moiety is a biotin residue and said capturing agent is avidin or streptavidin.

67. A method according to Claim 43, comprising:

20 (a) providing sensing member comprising a piezoelectric crystal having at least one sensing surface which carries capturing agents for binding to neutralizing agents;

(b) contacting the sample with neutralizing agents which can bind to the assayed molecules, said neutralizing agent being conjugated to an enzyme which can catalyze a reaction yielding an insoluble reaction product, for a time permitting binding of the neutralizing agent to the assayed molecules, to obtain a treated sample preparation;

25 (c) filtering the treated sample preparation through filtration system to filter out neutralizing agents unbound to the assayed molecules to obtain a filtrate essentially devoid of such unbound neutralizing agents;

30 (d) contacting said filtrate with the at least one sensing surface;

- (e) applying condition permitting the enzyme to catalyze the production of the insoluble reaction product;
- (f) inducing vibrations in the piezoelectric crystal and measuring resonance frequency thereof; and
- 5 (g) determining whether there occurred a decrease in resonance frequency after contact between the sensing surface and said filtrate or after application of said conditions, such decrease indicating presence of the assayed molecules in the sample.

68. A method according to Claim 67, wherein the assayed molecule is an 10 explosive molecule.

69. A method according to Claim 67 or 68, wherein said antibody is a monoclonal antibody.

70. A method according to Claim 69, wherein the assayed molecule is DNT or TNT and the monoclonal antibody is that designated herein as 5B3

15 71. A method according to any one of Claims 68-70, wherein said filtration system comprises immobilized residues or moieties of said assayed molecule.

72. A method according to any one of Claims 68-71, wherein said capturing agent is an immobilized antibody which binds a moiety of said enzyme, such binding not interfering with the catalytic activity of said enzyme.

20 73. A method according to any one of Claims 68-72, wherein said enzyme is selected from the group consisting of horseradish peroxidase, microperoxidase, alkaline phosphatase, glucoseoxidase and galactosidase.

74. A protein comprising an antigen-binding portion having two cooperating peptide sequences of Fig. 3A and Fig. 3B or an altered portion where at least one of the peptide sequences is an altered sequence, being a sequence of Fig. 3A or 3B in which one or more amino acid residue has been added, deleted or replaced by another amino acid residue, with the altered portion retaining substantially the same antigen-binding specificity as a non-altered portion.

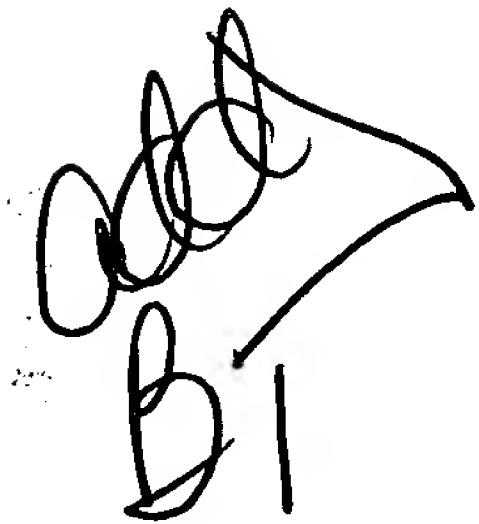
25 75. A protein according to Claim 74, being an antibody or a fragment of an antibody.

76. A protein according to Claim 75, being a monoclonal antibody.

77. A protein having an antigen-binding portion having substantially the same antigen binding specificity of the construct according to any one of Claims 74-76.

78. A protein according to Claim 77, being an antibody or a fragment of an antibody.

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